STUDIES ON THE PHYSIOLOGY OF CORALS

II. DIGESTIVE ENZYMES

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WITH

NOTES ON THE SPEED OF DIGESTION

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WITH SIX TEXT-FIGURES

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1. INTRODUCTION.

This paper deals with the work on the extracellular and intracellular digestive enzymes of corals. The structure of the gut will be discussed in the next paper, as it is more fitting that this should accompany a description of the processes of assimilation and excretion which follow those of digestion.

2. LITERATURE.

Apart from the observed fact that Madreporaria are able to digest with ease animal prey (Boschma (1925)), nothing has previously been known about the digestive enzymes of corals. There are, however, certain data on digestive processes in Coelenterates

generally. Since this phylum is, from the standpoint of feeding and digestion, extremely homogeneous—all its members feed in essentially the same manner, paralysing their prey with nematocysts and conveying it to the mouth by tentacles, and occasionally by means of cilia, and always taking the same type of food, namely animal prey—a short summary of the literature on the subject is desirable.

The occurrence of intracellular digestion in a variety of Coelenterates was first definitely established by Metschnikoff (1880, 1882), while Krukenberg (1886, which gives references to and summarizes previous work) stressed the importance in the actinians of extracellular digestion within the coelenteron. He maintained that gland-cells in the free margin of the mesenterial filaments secreted enzymes, and that digestion could only take place if the food lay against the filaments. He was unable, however, to find free enzymes in the coelenteron. Willem (1892, 1893) demonstrated, though in no very convincing manner, the presence of free protease, but not lipase or amylase in the coelenteron of actinians, but he agreed that subsequent digestion took place within the mesenterial filaments, thus reconciling the views of Metschnikoff and Krukenberg. Later (1894) he obtained similar results working on Siphonophores.

The work of Chapeaux (1893) in *Anemonia sulcata* is more convincing. He found a weak extracellular protease but no other enzymes after fibrin had been introduced into the coelenteron. He pointed out the necessity of a preliminary extracellular digestion if the fine particles and proteoses for subsequent intracellular digestion were to be formed. He also thought that the extracellular enzyme was secreted by gland-cells in the mesenterial filaments. He described an intracellular digestion of fats (olive oil), and a very powerful intracellular digestion of proteoses, a very weak action on starch, but none on algae.

Mesnil (1901), also working on actinians, was unable to find any extracellular digestion. He made tissue extracts which digested proteins over a wide range of hydrogen ion concentration, had a temperature optimum of between 36° and 45° C., and whose activity was destroyed at 64° C. They also digested, though to a much smaller extent, fats and starch. Fredericq (1878) had previously found a weak protease acting in neutral or alkaline media in extracts of actinian tissues. Jordan (1907), after feeding *Anemonia sulcata* with fibrin enclosed in bags of filter-paper, found that the fibrin was dissolved out by the action of an extracellular enzyme. He also followed the process of intracellular digestion, finding that a preliminary acid stage in the digestive vacuoles was followed by an alkaline one—an opinion in which he had later the support of Willem (1916). Haase (1916) also fed actinians on food enclosed in filter-paper, but found no digestion unless there was a hole in the envelope from which the enclosed food was not more than 2 mm. distant.

Miss Greenwood (Mrs. G. P. Bidder) (1888) drew attention to the probability of a preliminary extracellular digestion in *Hydra*, and Beutler (1924) has shown conclusively that an extracellular protease is secreted into the coelenteron which causes either the complete solution of flesh, or else its reduction to pieces small enough to be ingested intracellularly. The reaction in the coelenteron is alkaline, but within the digestive vacuoles it is first acid and later neutral. No other extracellular enzymes are present, but olive oil injected into the coelenteron is broken up into fine droplets by peristaltic movements and ingested, to be acted upon by intracellular lipase. Starch (but only if protein is also present, never if pure) and glycogen are ingested intracellularly. In a later paper (1926) similar findings were recorded for a variety of hydroids.

Roaf (1908) found that extracts of the mesenterial filaments of the actinian *Tealia crassicornis* showed digestive action on starch after 66 hours', and on maltose after 39 hours', incubation, but no action on glycogen, saccharose or lactose ; fibrin was digested over a wide range of hydrogen ion concentration, but best in neutral or alkaline media, with optimum conditions in N/20 sodium carbonate. Extracts of the filaments of *Actinia mesembryanthemum* gave similar results, except that no action on starch was found. The most accurate quantitative work on the digestive enzymes of Coelenterates is that of Bodansky and Rose (1922) on *Physalia arethusa* (Siphonophore), and *Stomolophus meleagris* (Scyphozoan), and of Bodansky (1924) on the actinian, *Metridium marginatum*. Experiments with tissue extracts of the siphons of *Physalia* and of the mesenterial filaments of *Stomolophus* revealed the presence in both of a protease with two optima, a " pepsin " with an optimum at pH 3·0 and a " trypsin " with one at pH 7·3, a weak lipase acting on the ester, ethyl butyrate, an amylase of moderate strength, very weak maltase and invertase, but no lactase or inulinase. Coelenteric fluid obtained from *Metridium* by draining and later cutting open the animals after feeding showed feeble action on protein (in neutral or slightly acid media) and starch, but this action was much weaker after the fluid had been filtered, and Bodansky thought that the enzymes might originate, at any rate in part, from the food. Extracts of the mesenterial filaments contained a very weak protease (again a " pepsin " and a more powerful " trypsin "), a weak lipase or esterase, an amylase and a maltase, but no lactase or invertase.

Finally Boschma (1925), working on the Madreporarian, *Astrangia danae*, found that an extract of the entire polyps partially dissolved crab-meat after 4 days, and he was able to demonstrate the presence in the digest of small amounts of amino-acids. He also followed the course of intracellular digestion within the absorptive cells of the mesenterial filaments by means of meat coloured with Indian ink, ammonia carmine or litmus, and showed that the reaction of the fluid in the coelenteron is neutral or slightly alkaline, whereas the digestive vacuoles are at first acid, and after about 2 days become alkaline.

It will be seen that in the coelenterates generally the body of evidence goes to show that there is a preliminary extracellular digestion of protein only, which is followed by intracellular digestion within the cells lining the coelenteron (especially in the mesenterial filaments). This intracellular digestion is not confined to proteins, but affects fats and, to an even smaller degree, some carbohydrates. There is a great lack of detailed quantitative work on digestive enzymes in the coelenterates.

3. MATERIAL AND METHODS.

Corals with large polyps and with as large an amount of tissue substance as possible were necessary for experiments on digestive enzymes. For work on the coelenteric fluid *Fungia danai* was used, and for tissue extracts *Lobophyllia corymbosa*, which occurred in large heads around Low Island and on Batt reef. The mesenterial filaments were too small to be removed singly from the polyps, so the latter were split longitudinally with bone forceps and the endodermal tissue scraped out by means of small brushes made of twisted wire. Extracts were made (unless otherwise stated) in filtered sea-water and were kept in the refrigerator, near freezing-point, until required, when they were filtered repeatedly until a clear filtrate was obtained. Toluol was invariably used as an antiseptic in both extracts and digests, and control experiments using boiled extract were always

set up. Owing to the presence of the buffering salts of sea-water, it was possible to prepare different pH media by adding acid or alkali followed by vigorous shaking. In the unavoidable absence of any facilities for thermostatic control, the digests were placed in a shady part of the aquarium, behind the laboratory, where the temperature remained comparatively constant in the neighbourhood of 25° C. Mrs. Yonge carried out the estimations for glucose, using McLean's blood-sugar method, acknowledgments being due to Mr. A. P. Orr for much valuable advice, and Mr. A. G. Nicholls assisted throughout in the collection of material, preparation of extracts and running of experiments.

4. THE COELENTERIC FLUID.

The fluid in the coelenteron of corals, being in free communication with the surrounding sea-water, is invariably colourless. Table I summarizes the results of pH estimations of the coelenteric fluid in five *Fungia* and one *Herpetolitha*, made when the animals were starving, and again 2 hours after feeding with pieces of mollusc flesh. The pH was estimated by removing each animal from the water, carefully pipetting out the contents of the coelenteron, and then mixing drops of this with indicator solutions on a white plate, the resultant colours being compared with those of the same indicator added to drops of solution of known pH value.

TABLE I.—*pH in the Coelenteron of* Fungia *and* Herpetolitha.

Animal.	pH in coelenteron of starved animal.	pH of water.	pH in coelenteron two hours after feeding.	pH of water.
Fungia 1	7·70	..	7·08	..
,, 2	7·75	..	6·89	..
,, 3	7·77	8·25	6·97	8·15
,, 4	7·85	..	6·85	..
,, 5	7·85	..	7·45	..
	Average—7·78		Average—7·05	
Herpetolitha	7·80	..	7·00	..

It will be noticed that in the case of *Fungia* the average pH in the coelenteron of the starved animals is 7·78, *i. e.* a little on the alkaline side of neutrality, and 0·47 lower than the pH of the water in the large glass tank in which the animals were kept. After feeding the average pH drops by 0·73 to 7·05—just on neutrality—and is now 1·10 lower than the surrounding water, the pH of which dropped 0·10 during the period owing to the effect of respiration by the animals, which were kept in the shade, where photosynthesis by the zooxanthellae would be slight. The conditions in the coelenteron of *Herpetolitha* were almost identical with those of *Fungia*. Boschma (1925) found that the pH of the coelenteric fluid in the (presumably) starving *Astrangia* was neutral or slightly alkaline, but Beutler (1927) states that in *Hydra* the pH is normally weakly alkaline, but that this rises to about 8·2 after feeding.

5. THE PROTEOCLASTIC ENZYMES.

(A) IN THE COELENTERIC FLUID.

Table II gives a summarized account of experiments on the enzymatic properties of the coelenteric fluid of *Fungia*. Fibrin was dissolved—more quickly by fluid removed from fed than from starving animals—and the resultant fluid gave a strong biuret reaction

after 21 days. Neither in the case of fibrin nor peptone was there any production even after 21 days of the amino-acids, tyrosine or tryptophane, tested for respectively by Millon's reagent and by bromine water after acidification. Further experiments with fibrin gave similar results, while casein was converted into polypeptides, as shown by absence of any precipitation following acidification one week after the experiment had been set up. Here, again, no amino-acids could be detected even after 57 days' incubation.

TABLE II.—*Presence of Protease in the Coelenteron of* Fungia.

Fluid pipetted out of coelenterons of 10 large *Fungia*. Made up to 20 c.c. in all by addition of sea-water, whole filtered. Fluid first from starved animals (0), then similar amount from same animals two hours after feeding with mollusc flesh (2).

Experiment.		Fibrin dissolved.	Tested after 21 days.		
Fluid.	Substrate.		Biuret.	Millon.	Bromine water.
5 c.c. (0)	5 c.c. 2% peptone	..	..	Negative	Negative.
	Control	..	..	,,	,,
5 c.c. (0)	0·25 grm. fibrin	6 days	Strong	,,	,,
	Control	Intact	Trace	,,	,,
5 c.c. (2)	5 c.c. 2% peptone	..	..	,,	,,
	Control	..	..	,,	,,
5 c.c. (2)	0·25 grm. fibrin	4 days	Strong	,,	,,
	Control	Intact	Trace	,,	,,

Details concerning the setting up and results of an experiment to determine the pH range of the activity of this extracellular protease are given in Tables III and IV. The rate of dissolution of fibrin was taken as the indication of the activity of the enzyme, and by this means the progress of digestion at the end of fifteen periods, between $2\frac{1}{2}$ and 57 days after the experiment was set up, was recorded. In Text-fig. 1 is shown a graph which records the progress of fibrin dissolution 5, 10 and 16 days after the setting up of the experiment. There are clearly two optima, one about pH 7·1 and the other about pH 8·7. It will be noted that the first of these corresponds almost exactly to the pH in the coelenteron *during* digestion. The significance of the second optimum will be discussed later. At the end of this experiment, *i. e.* after 57 days, E, H and K were tested for the presence of tyrosine with negative results in all cases.

TABLE III.—*pH Range for Action of Protease from Coelenteron of* Fungia.

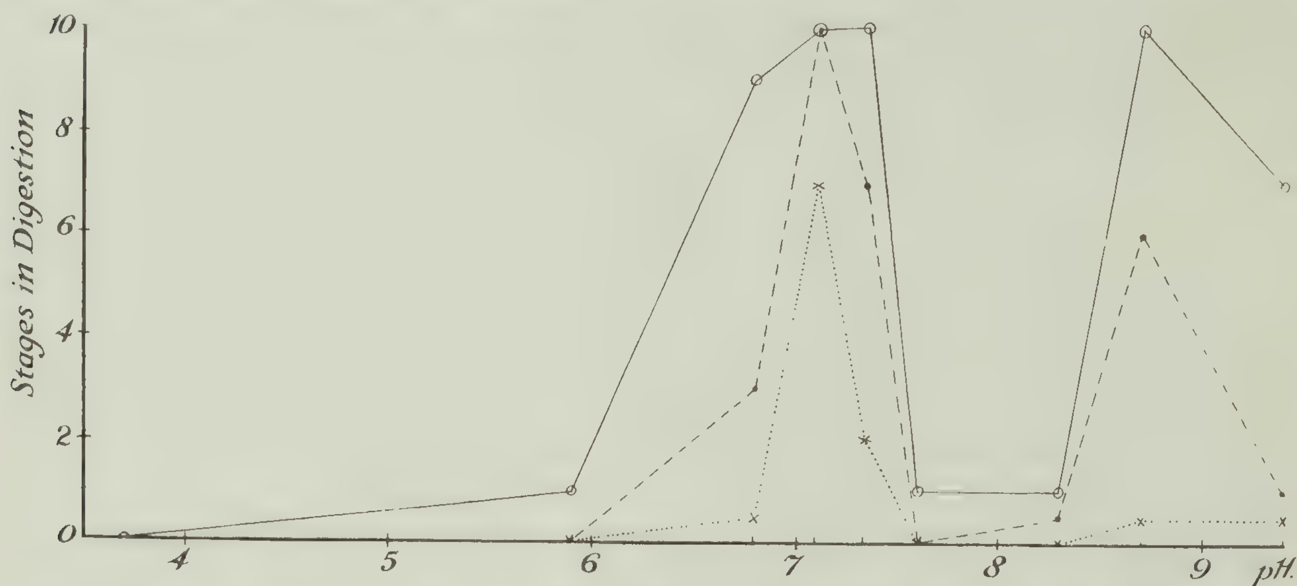
50 c.c. of fluid taken from 20 animals 2 hours after feeding with meat, filtered; 5 c.c. of fluid in each digest with acid or alkali made up to 10 c.c. with distilled water, 1 c.c. removed for pH determination. 0·05 grm. fibrin added to each.

No.	Fluid.	0·1 N. HCl.	0·1 N. NaOH.	Water.	Initial pH.
A	5 c.c.	0·15 c.c.	..	5·85	3·7
B	,,	0·10 c.c.	..	5·90	5·9
C	,,	0·05 c.c.	..	5·95	6·7
D	,,	0·02 c.c.	..	5·98	7·1
E	,,	..	..	6·00	7·35
F	,,	..	0·01 c.c.	5·99	7·60
G	,,	..	0·02 c.c.	5·98	8·30
H	,,	..	0·05 c.c.	5·95	8·70
I	,,	..	0·20 c.c.	5·80	9·40
K	,, boiled	..	..	6·0	7·35

TABLE IV. *Results of Experiment recorded in Table III.*

No.	Initial pH.	Days.														
		2½	3½	4	5	6	7	8	9	10	11	13	14	16	29	57
A	3·7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	5·9	0	0	0	0	0	0	0	0	0	0	0	? ½	1	7	8
C	6·7	0	0	0	½	1	1	1	2	3	5	7	8	9	10	10
D	7·1	1½	3	5	7	8	9	10	10	10	10	10	10	10	10	10
E	7·35	0	1	1½	2	2	3	3	5	7	8	9	10	10	10	10
F	7·6	0	0	0	0	0	0	0	0	0	0	0	½	1	7	9
G	8·3	0	0	0	0	0	0	0	0	½	1	1	1	1	5	9
H	8·7	0	0	½	½	1	1	1	3	6	7	9	10	10	10	10
I	9·4	0	0	0	½	½	½	½	½	1	1	2	5	7	8	8
K	7·35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Significance of numbers: 0 no dissolution of fibrin; ½ slightest trace dissolution; 1 9 various stages of dissolution; 10 complete dissolution.



TEXT-FIG. 1. Graphs showing dissolution of fibrin at various pH by the extracellular protease from *Fungia*. See Tables III and IV. .... after 5 days' digestion; - - - - - - - after 10 days' digestion; ——— after 16 days' digestion.

The filtered coelenteric fluid from *Fungia* thus contains an extracellular protease which can break down insoluble proteins to polypeptides—as shown by positive biuret tests but is incapable of converting them into amino-acids. It has two pH optima, the lower of which corresponds to the pH of the coelenteron during digestion.

(B) IN TISSUE EXTRACTS.

Extracts of the mesenterial filaments and other endodermal tissues of *Lobophyllia* contain powerful proteoclastic enzymes (it must be remembered that both the extra-cellular protease already described *and* any intracellular enzymes there may be will be present). Table V gives the results of experiments with peptone and fibrin, in both of which tryptophane (in the case of fibrin presumably due to the digestion of proteins in the extract containing the enzyme) was found after 3 days and tyrosine after 5 days. In the case of fibrin there were positive results with the biuret reaction after 1 day.

TABLE V. *Production of Amino-acids by Proteases of* Lobophyllia.

Tissue extracted for 7 days, filtered, adjusted to make 25% extract of original wet weight of tissue. pH of extract 6·8. Br. Bromine water. M. Millon's reagent.

Extract.	Substrate.	2 days.		3 days.		5 days.	
		Br.	M.	Br.	M.	Br.	M.
10 c.c.	10 c.c. 2% peptone	-		+		+	+
	Control	-			-		
10 c.c.	0·5 grm. fibrin			+		+	+
	Control		—	—	—		—

The pH range of the proteases was studied for a variety of substances, the results of work on the first of these, fibrin, being shown in Tables VI and VII, the former expressing the results in terms of amino-acid production as determined by Sörensen titrations, and the latter following the course of dissolution of fibrin in the same manner as in Table II.

TABLE VI.—*pH Range of Proteases acting on Fibrin.*

Tissue extracted for 7 days, filtered, adjusted to make 25% extract of original wet weight of tissue. 10 c.c. fluid used for each experiment with 10 c.c. distilled water and acid or alkali to make 25 c.c. in all. 3 c.c. removed for pH determination, 0·3 grm. fibrin added to each.

No.	HCl.	NaOH	Water.	Initial pH.	After 17 days, neutralized, then Sörenson titration with 0·1 N NaOH.
A	1·75 c.c. ·1 N.	..	3·25 c.c.	2·2	0·30 c.c.
B	1·0 ,,	..	4·0 ,,	2·8	0·30 ,,
C	0·4 ,,	..	4·6 ,,	3·25	0·40 ,,
D	0·15 ,,	..	4·85 ,,	3·7	0·80 ,,
E	0·12 ,,	..	4·88 ,,	4·0	1·08 ,,
F	0·10 ,,	..	4·90 ,,	4·6	1·10 ,,
G	0·5 c.c. ·01 N.	..	4·5 ,,	5·0	1·10 ,,
H	..	..	5·0 ,,	5·3	1·25 ,,
I	..	0·25 c.c. ·01 N.	4·75 ,,	5·8	1·225 ,,
J	..	0·5 ,,	4·5 ,,	6·2	1·40 ,,
K	..	1·0 ,,	4·0 ,,	6·7	3·30 ,,
L	..	1·5 ,,	3·5 ,,	7·4	1·85 ,,
M	..	2·0 ,,	3·0 ,,	8·05	1·75 ,,
N	..	0·5 c.c. ·1 N.	4·5 ,,	8·4	1·75 ,,
O	..	1·0 ,,	4·0 ,,	9·0	2·50 ,,
P	..	1·5 ,,	3·5 ,,	10·0 (about)	2·90 ,,

The results summarized in Tables VI and VII are shown graphically in Text-fig 2. It will be seen that dissolution of the fibrin and the formation of amino-acids by no means follow the same course. In the former there is a gradual rise from pH 2·2, reaching a sharp peak about pH 5·3, then falling again to attain a second optimum around pH 6·5, this optimum continuing, apart from a small depression between pH 7·5 and 9, to pH 10. Amino-acid production shows a general rise from acid to alkaline conditions with two optima, one a sharp peak at pH 6·7 and the other in the neighbourhood of pH 10·0, beyond which the experiment did not continue.

The problem now is to interpret these apparently conflicting results. In the first place it must be remembered that an extracellular protease and one or more intracellular proteases are being dealt with. It will be convenient to call the extracellular protease enzyme A. This (see Text-fig. 1) converts proteins into proteases, but *not* into amino-acids; it has two optima, one about pH 7·1 and the other between pH 8·7 and 9·0. Since

the pH in the coelenteron, though weakly alkaline in the starving animal (conditions are the same in *Lobophyllia* as in *Fungia*), is *never* as high even as sea-water (8·25 approximately), and falls to about neutrality after feeding, it follows that the second optimum has a theoretical but *not* a practical interest. It is to be explained probably in the same way as the second pH optimum of trypsin in its action on fibrin. This lies at pH 11·3, and has recently been shown by Vonk and Heyn (1929) to be due to the effect of the hydrogen ion concentration on the substrate, fibrin.

TABLE VII.—*Dissolution of Fibrin by Proteases in Different pH.*

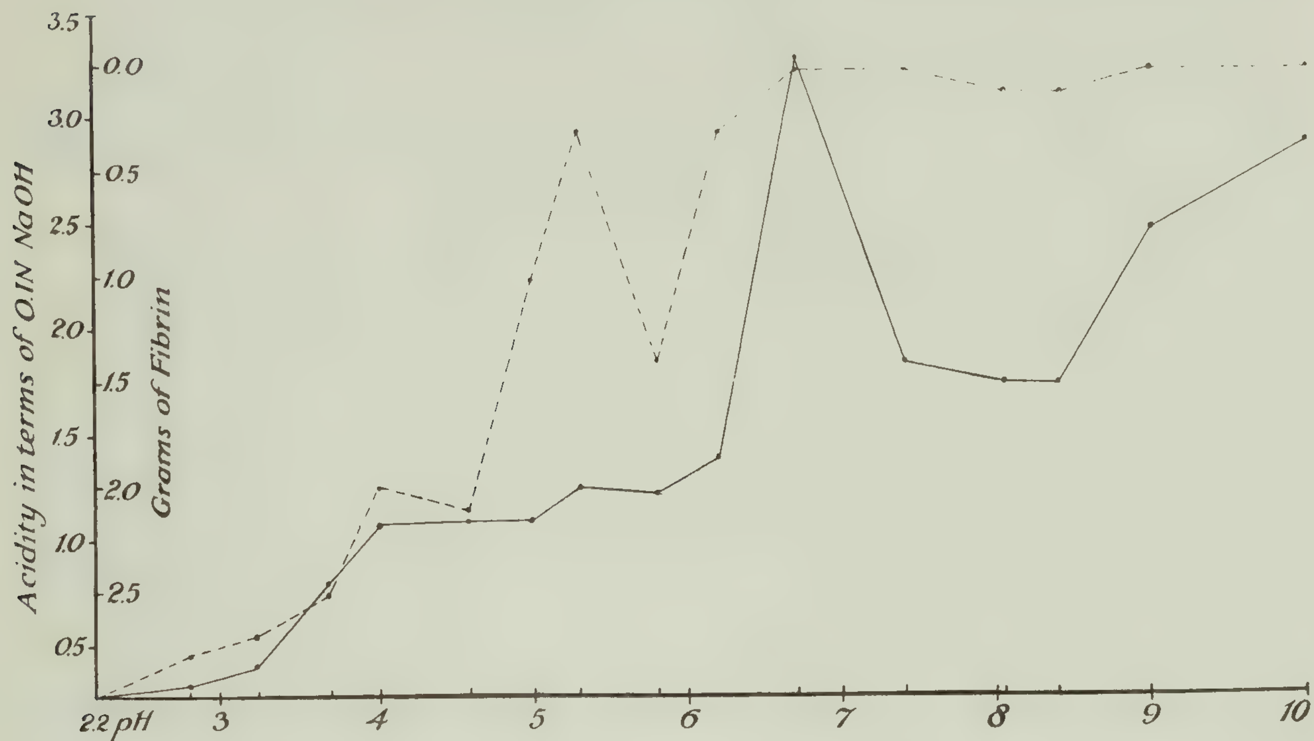
In this table the course of fibrin digestion over the 17 days that the experiment continued is shown in terms of the rate of dissolution of the fibrin. The figures have the same significance as in Table II.

No.	Initial pH.	Days						Weight of dried fibrin after 17 days.
		5.	7.	9.	11.	13.	16.	
A	2·2	0	0	0	0	0	0	0·30 grm.
B	2·8	0	0	0	0	0	0	0·28 ,,
C	3·25	0	0	0	0	0	0	0·27 ,,
D	3·7	0	0	0	0	0	0	0·25 ,,
E	4·0	0	0	0	0	0	0	0·20 ,,
F	4·6	0	0	0	0	0	0	0·21 ,,
G	5·0	0	0	0	½	1	2	0·10 ,,
H	5·3	0	0	½	2	3	9	0·03 ,,
I	5·8	0	0	0	0	½	1	0·14 ,,
J	6·2	0	0	½	1	2	6	0·03 ,,
K	6·7	0	0	3	6	9	10	0 ,,
L	7·4	0	0	3	6	8	10	0 ,,
M	8·05	0	0	0	2	5	9	0·01 ,,
N	8·4	1	2	7	8	9	9	0·01 ,,
O	9·0	1	7	9	10	10	10	0 ,,
P	10·0	6	8	9	10	10	10	0 ,,

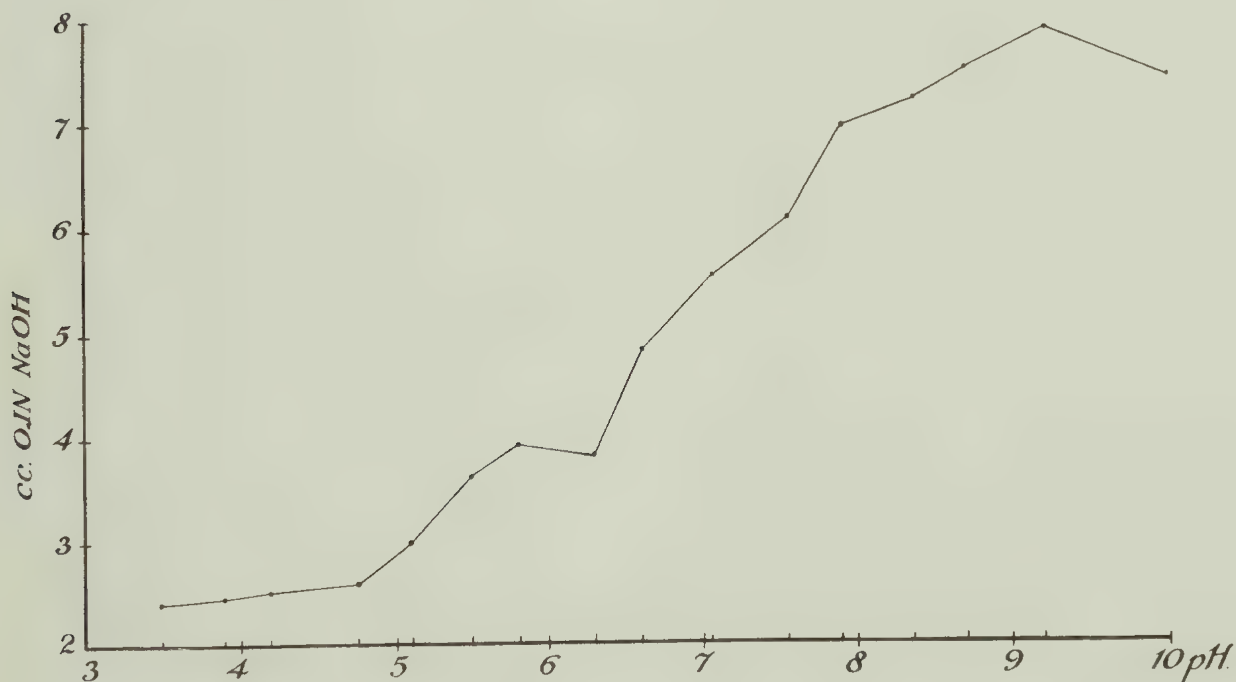
Turning now to the enzymes in the extracts. The graph for amino-acid production in Text-fig. 2 shows a general rise from pH 2·2 to pH 10 with the exception of a sharp peak at pH 6·7. It seems reasonable to suppose that this peak is caused by the action of enzyme A, which, by converting the insoluble fibrin into soluble polypeptides, will obviously assist the intracellular protease -hereafter termed enzyme B –in its digestive action. Enzyme B is an intracellular protease which breaks down polypeptides and, in view of the dissolution of fibrin and the formation of amino-acids between pH 7·6 and 8·3 when enzyme A is practically without action (see Text-fig. 1), also proteins, to amino-acids.

But difficulties still remain. If A and B are the only proteoclastic enzymes present, the graph of fibrin dissolution in Text-fig. 2 should follow much the same course as the graph of amino-acid production. With the exception of the sharp peak about pH 5·3, it does so. The graph of amino-acid production shows a very small peak at the same place. As will be shown later, there is no evidence that the pH of the tissues or digestive vacuoles falls at any stage even as low as 6·0. Assuming that we are dealing with a third enzyme, C, then the probability is that this originates in the algal cells—a matter which will be discussed in the section of this paper which deals with the enzymes from the zooxanthellae.

The influence of hydrogen ion concentration on the breaking down of gelatin by the



TEXT-FIG. 2.—Graphs showing dissolution of fibrin and production of amino-acids at various pH by the proteases from tissue extracts of *Lobophyllia*. See Tables VI and VII. ------- dissolution of fibrin ; ———— production of amino-acids.



TEXT-FIG. 3.—Graph showing production of amino-acids at various pH by the proteases from tissue extracts of *Lobophyllia*. See Table VIII.

protease was investigated. Details of this experiment are given in Table VIII, the results being expressed graphically in Text-fig. 3.

TABLE VIII.—*pH Range for Production of Amino-acids from Gelatin by Proteases.*

Tissue extracted for 7 days, filtered, adjusted to 25%; 10 c.c. fluid used for each experiment with 10 c.c. 10% gelatin and acid or alkali with water to make 25 c.c. in all; 3 c.c. removed for pH determinations.

No.	0·1 N. HCl.	0·1 N. NaOH.	Water.	Initial pH.	After 14 days, neutralized, then Sörenson titration 0·1 N. NaOH used.
A	5 c.c.	..	..	3·5	2·40 c.c.
B	4 ,,	..	1 c.c.	3·9	2·45 ,,
C	3 ,,	..	2 ,,	4·2	2·50 ,,
D	2 ,,	..	3 ,,	4·75	2·58 ,,
E	1 ,,	..	4 ,,	5·1	2·98 ,,
F	0·4 c.c.	..	4·6 c.c.	5·5	3·65 ,,
G	0·2 ,,	..	4·8 ,,	5·8	3·95 ,,
H	..	..	5 ,,	6·3	3·86 ,,
I	..	0·25 c.c.	4·75 ,,	6·6	4·87 ,,
J	..	0·5 ,,	4·5 ,,	7·05	5·55 ,,
K	..	0·7 ,,	4·3 ,,	7·55	6·1 ,,
L	..	1·0 ,,	4·0 ,,	7·9	7·0 ,,
M	..	1·5 ,,	3·5 ,,	8·35	7·25 ,,
N	..	2·0 ,,	3·0 ,,	8·7	7·55 ,,
O	..	2·5 ,,	2·5 ,,	9·2	7·95 ,,
P	..	3·0 ,,	2·0 ,,	10	7·48 ,,

Comparing the graph in Text-fig. 3 with that for amino-acid production in Text-fig. 2, it will be noted that there is the same gradual increase in the production of amino-acids from the acid to the alkaline end of the pH range, with a definite optimum at pH 9·2, but with no peak about pH 6·7. This is explained by the fact that the gelatine is dispersed throughout the fluid, thus exposing the maximum surface to the action of the enzyme—and enzyme action is essentially a surface reaction. Thus the action of extracellular protease A does not provide the same help over its particular pH optima as in the case of fibrin, where only a very limited surface is exposed to the action of enzymes, and where the assistance of enzyme A in increasing the surface of the substrate is clearly of the first importance. There is a minor peak on the graph in Text-fig. 3 about pH 5·8. This, again, though it is too small for much emphasis to be laid upon it, may be caused by the problematic plant protease C.

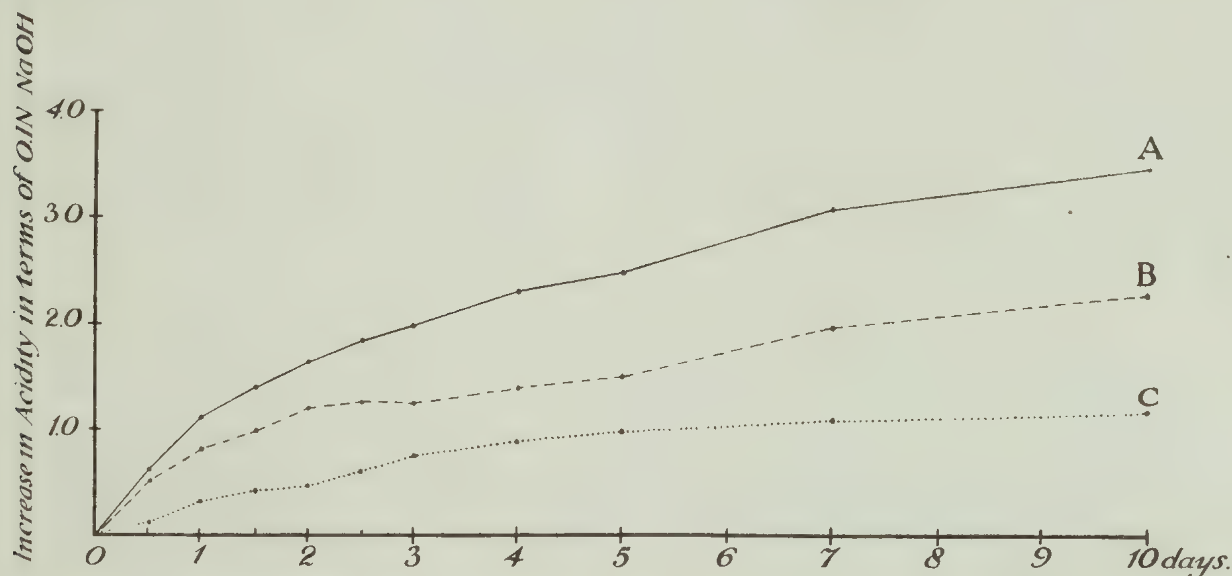
The velocity of reaction of enzyme B and the relative production of "free acidity" and "formaldehyde acidity" were studied, with the results shown in Table IX and in Text-fig. 4. The velocity of reaction as shown by increase in total acidity is considerable in the early stages of digestion, gradually falling off after 2 to 3 days, but still increasing slightly after 10 days. As quoted by Wigglesworth (1928), Cole has noted the interesting distinction between peptic and tryptic digestion when they are followed by Sörensen's formaldehyde titration. The "free acidity" which has to be neutralized if the digest is to be brought to an arbitrary pH—say the point at which phenolphthalein turns pink—increases as digestion proceeds; so does the "formaldehyde acidity," *i. e.* the acidity due to the setting free of the acid radicle of the amino-acids following the addition of neutralized formaldehyde. In the case of trypsin digestion the increase in "free acidity" is greater than that of "formaldehyde acidity" during the earlier stages of digestion, but the former soon decreases, while the latter continues to increase and passes it. In peptic digestion the "free acidity" at all stages of digestion is far greater than the "formaldehyde acidity." Wigglesworth found that the protease from the mid-gut of the cockroach was

of the tryptic type. As shown in Text-fig. 4, the protease of *Lobophyllia* behaves unlike either trypsin or pepsin, since at no period is the increase in "free acidity" greater than that of "formaldehyde acidity," although apart from this it agrees with the conditions found in tryptic digestion.

TABLE IX.—*Velocity of Reaction of Proteases on Gelatin; Formation of "Free Acidity" and "Formaldehyde Acidity."*

Tissue extracted for 7 days, filtered, adjusted to 25%, 80 c.c. each of extract and of 10% gelatin made up to 220 c.c. with water and pH adjusted to 7·0. 20 c.c. removed at stated intervals, titrated directly with 0·1 N. NaOH with phenolphthalein as indicator, and again after addition of 10 c.c. neutral formaldehyde.

Acidity.	Initial.	Increase in acidity after— (hours).									
		½.	1.	1½.	2.	2½.	3.	4.	5.	7.	10.
"Free"	0·5 c.c.	0·1	0·3	0·4	0·45	0·6	0·75	0·9	1·0	1·1	1·2 c.c.
"Formaldehyde"	2·3 ,,	0·5	0·8	1·0	1·2	1·25	1·25	1·4	1·5	2·0	2·3 ,,
Total	2·8 ,,	0·6	1·1	1·4	1·65	1·85	2·0	2·3	2·5	3·1	3·5 ,,



TEXT-FIG. 4.—Graphs showing velocity of reaction of the proteases from tissue extracts of *Lobophyllia* as demonstrated by the production of amino-acids from gelatin, also the increase in "formaldehyde" and "free" acidity. See Table IX. .... "free" acidity; - - - - - - - "formaldehyde" acidity; ———— total acidity.

It has been shown by the researches of Waldschmidt-Leitz and co-workers (1929) that the simplest types of proteoclastic enzymes are the peptidases, which are specific for the hydrolysis of dipeptides exclusively, and are quite incapable of attacking the polypeptides, such as peptone, protamine or histone, which are hydrolysed by trypsin. Peptidase can be separated from trypsin by fractional adsorption with alumina as demonstrated by Waldschmidt-Leitz and Harteneck (1925). Their procedure was followed, using the tissue extract of *Lobophyllia*. Thirty-two grammes wet weight of tissue was extracted for 7 days in 10 c.c. of glycerine; the extract was then filtered, made up to 12 c.c., and 1 c.c. of a buffer solution of pH 4·6 added. This was shaken up with 2·5 c.c. of freshly prepared aluminium hydroxide, allowed to stand for 2 minutes and then centrifuged. This procedure was repeated twice on the supernatant fluid, which was brought to pH 8·0 and called solution A. The precipitate of aluminium hydroxide in

the original mixture was then extracted for 3 hours with 10 c.c. of N/25 ammonia in 18% glycerine, the mixture centrifuged and the supernatant fluid also brought to pH 8·0. This was called solution B. Both A and B were then brought to 40 c.c. by the addition of distilled water, and the experiments outlined in Table X set up :

TABLE X. *Identification of Peptidase in Extracts of* Lobophyllia.

Solution.	Substrate.	Fibrin after 31 days.	Bromine water.
10 c.c. A	0·1 grm. fibrin	Gone	..
	Control	Intact	..
10 c.c. B	0·1 grm. fibrin	,,	..
	Control	,,	..
10 c.c. A	10 c.c. 0·2% glycyl-d-tryptophane	..	Negative.
	Control	..	,,
10 c.c. B	10 c.c. 0·2% glycyl-d-tryptophane	..	Positive after 5 days.
	Control	..	Negative.

As a result of the adsorption of the peptidase by the aluminium hydroxide, the protease left in solution A is capable of breaking down fibrin, but has no action on the dipeptide, glycyl-d-tryptophane. On the contrary the enzyme adsorbed by the alumina in solution B has no action on fibrin, but hydrolyses the dipeptide with the formation of tryptophane after 5 days. There is thus evidence of the presence of both a tryptic-like enzyme and of a simpler peptidase, the two probably working in conjunction within the tissues.

The glycyl-d-tryptophane employed was prepared in the Biochemical Laboratory, Cambridge, and was kindly sent to me by Dr. J. Needham.

6. THE LIPOCLASTIC ENZYMES.

Tests with the fluid from the coelenteron of starved *Fungia* and again after feeding with olive oil failed to demonstrate the presence of any extracellular lipase.

With tissue extracts of *Lobophyllia* an emulsion of olive oil, to which were added phenol red and as much sodium carbonate as was needed to turn the fluid a distinct pink, showed the presence of fatty acids after 12 days, as demonstrated by a change in colour from pink to yellow.

Table XI gives the results of quantitative tests :

TABLE XI.—*Presence of Lipase and Esterase in Tissue Extracts of* Lobophyllia.

Tissue extracted for 2 and 7 days respectively for two experiments.

Extract.	Substrate.	pH.	Time.	Titration with 0·05 N. NaOH.
20 c.c. 20%	20 drops olive oil emulsion	6·5	13 days	3·30
	Control	,,	,,	1·70
				1·60 c.c. difference.
25 c.c. 25%	5 c.c. 20% neutralized methyl acetate	6·8	19 ,,	3·90
	Control	,,	,,	2·70
				1·20 c.c. difference.

A lipase and an esterase—possibly the same enzyme—are therefore present intracellularly, but in very small quantities. No work on the optimum pH was carried out owing to the recognized unsatisfactory results in the case of all lipases.

7. SUCROCLASTIC ENZYMES.

As shown by negative tests, using the fluid from the coelenteron of starving *Fungia* and that from animals fed with a variety of carbohydrates, there are no extracellular sucroclastic enzymes.

Tissue extracts from *Lobophyllia* were tested for the presence of a variety of sucroclastic enzymes with the results shown in Table XII. Of the carbohydrates and glucosides tested, only starch and glycogen (the latter very slowly) are split up with the formation of reducing sugars. In both cases the end-products of digestion as shown by the phenylhydrazine test consisted of glucose. Maltose was never identified at any stage in digestion.

TABLE XII.—*Specificity of Sucroclastic Enzymes in Tissue Extracts of* Lobophyllia.

Tissue extracted for 7 days, filtered, adjusted to 25%. pH of fluid 5·3.

Extract.	Substrate.	Time.	Result.
15 c.c.	15 c.c. 1% starch	3 days	Reduction with Fehling's solution.
	Control	,,	No reduction.
15 ,,	15 c.c. sat. sol. glycogen	13 days	Reduction with Fehling's solution.
	Control	,,	No reduction.
15 ,,	15 c.c. 5% sucrose	32 days	,,
	Control	,,	,,
10 ,,	10 c.c. 1% raffinose	,,	,,
	Control	,,	,,
15 ,,	15 c.c. 15% amygdalin	,,	,,
	Control	,,	,,
10 ,,	10 c.c. 0·5% pectin	,,	,,
	Control	,,	,,
10 ,,	0·1 grm. cellulose	,,	,,
	Control	,,	,,
10 ,,	10 c.c. 2% maltose	,,	,, with Barfoed's solution.
	Control	,,	,, ,, ,,
10 ,,	10 c.c. 2% lactose	,,	,, ,, ,,
	Control	,,	,, ,, ,,

TABLE XIII.—*pH Range for Action of Amylase.*

Tissue extracted for 7 days, filtered, adjusted to 25%; 10 c.c. of extract from each experiment with 10 c.c. 1% starch solution and acid or alkali with water to make 25 c.c. in all; 3 c.c. removed in every case for pH determination.

No.	HCl.	NaOH.	Water.	Initial pH.	Percentage glucose after 4 weeks as determined by McLean's method.
A	1·3 c.c. ·1 N.	..	3·7 c.c.	2·1	0
B	1·0 ,, ,,	..	4·0 ,,	2·7	0
C	0·5 ,, ,,	..	4·5 ,,	3·2	0·312
D	0·25 ,, ,,	..	4·75 ,,	3·5	0·413
E	0·15 ,, ,,	..	5·85 ,,	3·8	0·413
F	0·1 ,, ,,	..	4·90 ,,	4·6	0·488
G	0·5 ,, ·01 N.	..	4·50 ,,	5·2	0·238
H	..	..	5·0 ,,	5·6	0·156
I	..	1·0 c.c. ·01 N.	4·0 ,,	6·4	0·138
J	..	1·5 ,, ,,	3·5 ,,	6·85	0·125
K	..	2·0 ,, ,,	3·0 ,,	7·4	0·088
L	..	3·0 ,, ,,	2·0 ,,	8·0	0·069
M	..	4·5 ,, ,,	0·5 ,,	8·6	0·044
N	..	1·0 ,, ·1 N.	4·0 ,,	9·1	0
O	..	1·5 ,, ,,	3·5 ,,	10·0 (about)	0

In the attempt to discover the properties of these enzymes and to find out whether they were actually the same enzyme or not, the temperature of destruction was first studied; in both cases, however, it was found that destruction took place between 60° C. and 65° C. The influence of hydrogen ion concentration in the activity of the enzymes was next studied, the results being shown in Table XIII and Text-fig. 5 for the amylase, and Table XIV and Text fig. 6 for the glycogenase.

TABLE XIV.—*pH Range for Action of Glycogenase.*

Experimental details as in Table XIII; saturated solution of glycogen instead of starch.

No.	0·1 N. HCl.	0·1 N. NaOH.	Water.	Initial pH.	Percentage glucose after 6 weeks as determined by McLean's method.
A	1·5 c.c.	..	3·5 c.c.	2·5	0
B	1·0 ,,	..	4·0 ,,	3·0	0
C	0·5 ,,	..	4·5 ,,	3·45	0·226
D	0·3 ,,	..	4·7 ,,	4·2	0·244
E	0·275 c.c.	..	4·725 c.c.	4·6	0·231
F	0·225 ,,	..	4·775 ,,	5·2	0·200
G	0·2 ,,	..	4·80 ,,	5·75	0·188
H	0·125 ,,	..	4·875 ,,	6·1	0·094
I	0·1 ,,	..	4·90 ,,	6·55	0·131
J	..	..	5·0 ,,	7·3	0·019
K	..	0·03 c.c.	4·97 ,,	7·7	0·025
L	..	0·05 ,,	4·95 ,,	8·1	0
M	..	0·1 ,,	4·90 ,,	8·3	0
N	..	0·25 ,,	4·75 ,,	8·5	0
O	..	0·5 ,,	4·50 ,,	9·2	0
P	..	1·0 ,,	4·0 ,,	10·0 (about)	0

A study of the graphs shows at once that a well-pronounced optimum occurs in both cases at the same point—about pH 4·2. Now at this low pH protease B is hardly active at all, while the pH of the tissues or digestive vacuoles never falls below 6·0. The possibility that, as in the case of protease C, these enzymes might come in whole or in part from the zooxanthellae, led to experiments being carried out with a coral which contains no zooxanthellae, *Dendrophyllia nigrescens*. This coral was dredged from 16 fathoms, and the supply was, unfortunately, too limited to permit of more than qualitative experiments, the results of which are shown in Table XV:

TABLE XV.—*Enzymes in* Dendrophyllia.

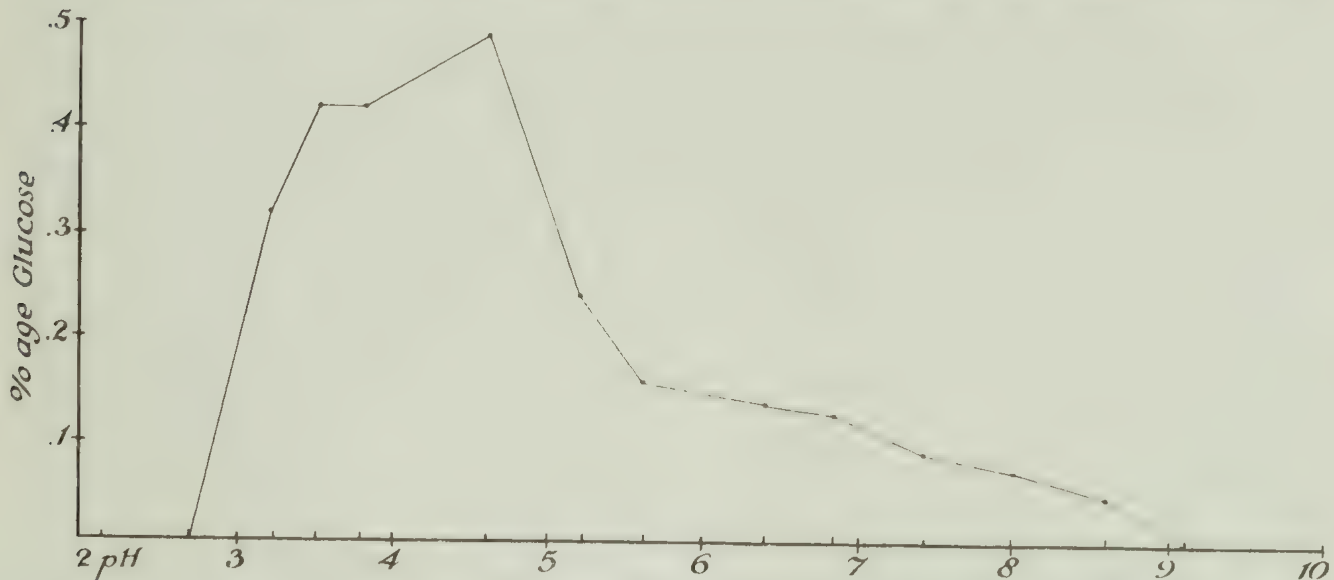
25 polyps of *Dendrophyllia nigrescens* ground up and extracted for 4 days, filtered; total fluid made up to 60 c.c.

Extract.	Substrate.	Time.	Results.
10 c.c.	10 c.c. 1% starch	40 days	No reduction with Fehling.
	Control	,,	,, ,, ,,
10 ,,	10 c.c. saturated solution glycogen	15 days	Reduction with Fehling.
	Control	,,	No reduction with Fehling
10 ,,	0·07 grm. fibrin	12 days	Fibrin breaking up.
	Control	,,	,, intact.

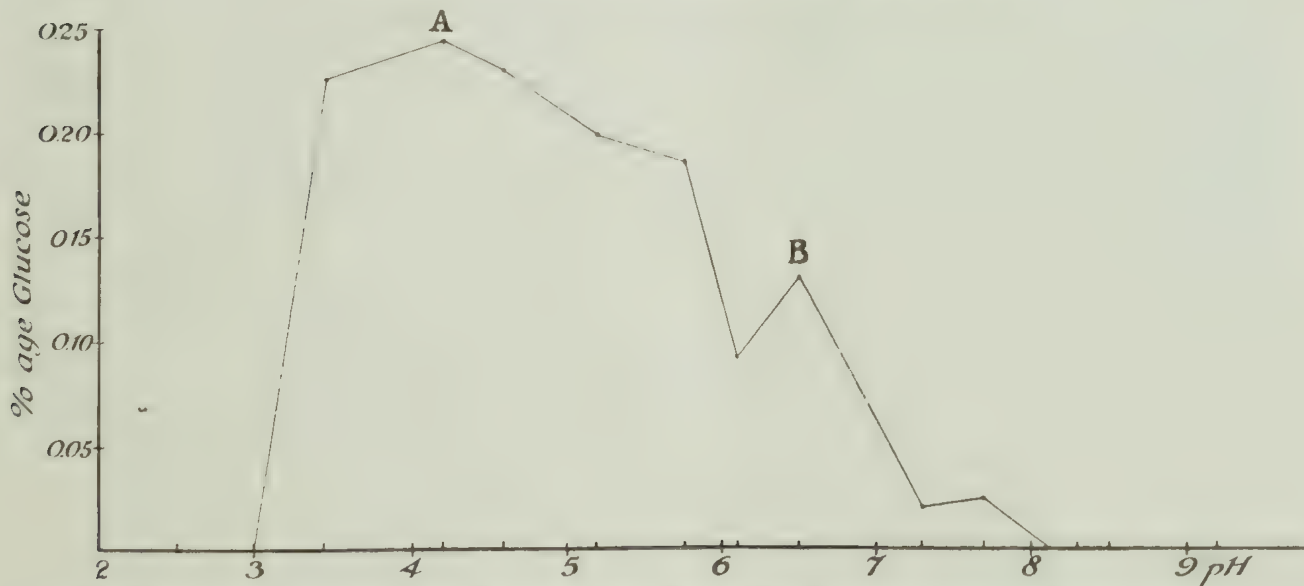
It appears, therefore, that extracts of corals without zooxanthellae are unable to split starch, although they contain a glycogenase and, of course, a protease. It was possible

later to obtain confirmation of this lack of amylase, using extracts of *Dendrophyllia manni* obtained at Honolulu.

Returning to the consideration of Text-figs. 5 and 6, if the evidence obtained from *Dendrophyllia* is valid for *Lobophyllia* (and there is no reason why this should not be so),



TEXT-FIG. 5.—Graph showing digestion of starch at various pH by amylase from tissue extracts of *Lobophyllia*, *i. e.* from Zooxanthellae. See Table XIII.



TEXT-FIG. 6.—Graph showing digestion of glycogen at various pH by glycogenase from tissue extracts of *Lobophyllia*. A, optimum due to action of amylase from zooxanthellae; B, optimum due to glycogenase from *Lobophyllia*. See Table XIV.

then the graph in Text-fig. 5 shows the result of the activity of an amylase from the zooxanthellae, and as such will be discussed in the next section. In Text-fig. 6 there are two enzymes to be considered, for, as will be shown a little later, the amylase of the algae can split up glycogen. This fact accounts for the presence of identical optima about pH 4·2, but in the case of glycogen there is a second smaller but well-pronounced optimum probably a little below pH 6·5. This, agreeing as it does with the pH in the digestive vacuoles during the early stages of intracellular digestion, is clearly the optimum

of the glycogenase for the coral. This is in general agreement with the optimum pH for the working of the sucroclastic enzymes of other invertebrates, *e.g.* the amylases of *Holothuria* 6·0 (Oomen (1926)), of *Caudina* 6·3 (Sawano (1928)), *Astacus* 5·6 (Wiersma and Veen (1928)), *Sabella* 6·8 (Nicol (1930)), *Ostrea*, style 5·9 and digestive diverticula 5·5 (Yonge (1926)).

Experiments were carried out to determine whether or not the zooxanthellae could be digested by extracts containing the digestive enzymes of corals. The algae were obtained by scraping off the edge zone tissue from *Lobophyllia* and then shaking the fragments of tissue in sea-water, when the algae were freed in large numbers. Experiments using strong extracts of the tissues of *Lobophyllia* and of *Euphyllia glabrescens* with a series of controls with boiled extract and with sea-water alone failed to indicate any digestion of algae even after 40 days. In view of the absence of any cellulose-splitting enzyme and the general lack of the means of digesting plant matter this result was to be expected. Chapeaux (1893) reports similar findings for the actinian *Anemonia sulcata*, adding (p. 159) that the zooxanthellae can even be cultivated in " le liquide fermentifère des Actinies."

8. ENZYMES FROM ZOOXANTHELLAE.

Evidence has been presented indicating the presence in the tissue extracts of *Lobophyllia* of enzymes originating, not in the coral, but in the zooxanthellae which crowd their tissues. The question was put to the test by making extracts, not of the digestive region, but of the outer, edge-zone tissues of *Lobophyllia*, the greatest care being taken to avoid contamination with digestive tissues. The polyps of *Lobophyllia* are so large and the edge-zone tissue so fleshy that this was not difficult. The material for extraction consisted of a dark brown mass containing little actual animal tissue, but much mucus and great numbers of zooxanthellae. Details and results of the experiments are given in Table XVI :

TABLE XVI. *Enzymes from Edge-Zone Tissue of* Lobophyllia.

A, 6·5 grm. tissue extracted 4 days, filtrate made up to 60 c.c. B, 6 grm. extracted 3 days, filtrate to 60 c.c. C, 7 grm. extracted 3 days, filtrate to 20 c.c.

Extract.	Substrate.	Time.	Result.
10 c.c. A .	10 c.c. 1% starch . .	10 days .	Clear reduction with Fehling.
	Control	,, .	No ,, ,, ,,
10 ,, A .	10 c.c. sat. sol. glycogen .	,, .	Slight ,, ,, ,,
	Control	,, .	No ,, ,, ,,
10 ,, B .	10 c.c. 1% starch .	18 days .	Clear ,, ,, ,,
	Control	,, .	No ,, ,, ,,
10 ,, A .	0·05 grm. fibrin . .	21 days .	No dissolution of fibrin.
	Control	,, .	,, ,, ,,
10 ,, B .	0·05 grm. fibrin . .	30 days .	,, ,, ,,
	Control	,, .	,, ,, ,,
10 ,, B .	5 c.c. emulsion olive oil pink with Na_2CO_3 and phenol red .	10 days .	Yellow.
	Control	,, .	Still pink.
10 ,, C .	5 c.c. emulsion olive oil pink with Na_2CO_3 and phenol red .	12 days .	Yellow.
	Control	,, .	Still pink.

Both starch and glycogen are digested by extracts of the edge-zone—*i. e.* by extracts of the zooxanthellae. It is not surprising that the plant amylase should also attack glycogen, a polysaccharide closely allied to starch, and this confirms the impression gained after examining Text-figs. 5 and 6. Thus whereas extracts of the digestive tissues of *Dendrophyllia* fail to show any action on starch (the coral glycogenase having, apparently, greater specificity of action than the plant amylase), extracts of the non-digestive tissue of *Lobophyllia* digest it freely. A lipase is present in the extracts but, since intracellular lipases are universal in animal tissues, there is not the same reason for attributing this to the algae. In view of the fact (which will be discussed in paper IV in this series) that the algae contain much fat, a part at least of the lipase probably originates in them. Fibrin was not obviously attacked in the experimental period. There were, however, only small indications of action by a possible plant protease shown in Text-fig. 2, while the pH at which it acted (5·3) was well between the pH of the extract of the edge zone—about 6·5. There was, unfortunately, no time to carry out further experiments at a lower pH.

The amylase of the zooxanthellae has an optimal pH at about 4·2, the possible protease one at about pH 5·3—both well on the acid side of neutrality. It is interesting to compare these optima with those obtained for other plant amylases and proteases, especially of lower groups, a few of which are shown in Table XVII :

TABLE XVII.—*pH Optima for Plant Amylases and Proteases.*

Amylase.			Protease.		
Plant.	Optimal pH.	Authority.	Plant.	Optimal pH.	Authority.
Aspergillum	4·8	See Waksman and Davison (1926)	Aspergillum	5·1	See Waksman and Davison (1926).
Malt	4·4 – 4·5	Ditto	Malt	3·7 – 4·2	Ditto.
Leaves	5·0 – 5·5	Sjöberg (1922)	Yeast	5·0	Willstätter and Grassmann (1926).
Zooxanthellae	4·2	This paper	Zooxanthellae	5·3	This paper.

The general tendency for these plant enzymes to find their pH optimum in an acid medium in the same neighbourhood as the pH optima for the enzymes for the zooxanthellae will at once be seen.

9. SPEED OF DIGESTION.

Many observers have been impressed by the almost invariable absence of food from the coelenteron of corals examined in the pickled state or immediately after collection during the daytime (see Boschma, 1926). This fact has been produced as evidence that corals do not feed on plankton, or only to a minor extent. It has already been shown in the first paper of this series that corals *can* capture plankton. In the course of these investigations food was very seldom found in coral polyps, but since corals feed almost exclusively at night when alone the polyps of the great majority expand, and when alone there is abundant plankton, it is clearly essential to possess data on the nature and speed of digestion within the coelenteron before any conclusions can be drawn from the lack of food in the coelenteron during the day. Moreover, as Boschma (1924) has pointed out, some loss of food from the coelenteron of fixed corals may take place as a result of contraction.

Boschma (1925) has demonstrated that the progress of digestion of living prey can be followed by placing living plankton (copepods), stained with neutral red, on the mesenterial filaments of corals (*Astrangia*), and he found that the soft parts were almost completely digested out of a copepod after 3 hours, the red colour of the flesh appearing within the absorptive zone of the mesenterial filaments. In the present work similar experiments were carried out, using the polyps of *Euphyllia*, which were split in half to expose the mesenterial filaments. Living plankton organisms stained with neutral red were placed on the mesenterial filament, which quickly closed over them, so that the prey was almost completely obscured after 10 minutes. The filaments were continually in motion, one replacing another as soon as the first was gorged with food. Details of these experiments are summarized in Table XVIII.

TABLE XVIII. *Digestion of Plankton by Mesenterial Filaments of* Euphyllia.

	Oikopleura.	Copepod, 3 mm.	Sagitta, 8 mm.
½ hour	Colour passing into filaments	Colour passing into filaments	Colour passing into filaments.
1½ hours	Partially digested; filaments red immediately round food	Partially digested; many filaments reddish	Partially digested; many filaments reddish.
2½ ,,	Practically all digested	Body tissues partially gone	Three-quarters digested.
3 ,,	All digested	Digestion proceeding	Digestion proceeding.
14 ,,	..	Almost all digested; shell detached	Completely digested.

Further evidence of the rapidity of this extracellular digestion of protein in the stomach was furnished by experiments using coagulated masses of the blood of the cat shark which were eagerly taken by *Fungia*. After one hour it was found that some of the corpuscles were irregular and lobed, after 2 hours they were practically all irregular in shape, while after 4 hours they were greatly reduced in numbers, and all remaining were half digested, greatly reduced in size and very irregular.

The process of intracellular digestion can be followed after feeding with food stained with neutral red or brom thymol blue, and results in general agreement with those of Boschma (1925) were found, namely that the pH of the digestive vacuoles is first about 6·4 (red with neutral red and green with brom thymol blue), and after about 2 days becomes more alkaline, probably not above 7·5 (brown with neutral red and bluish green to blue with brom thymol blue).

These experiments demonstrate the course and rapidity of the extracellular digestion in the coelenteron, but afford no satisfactory data regarding the speed of digestion under normal conditions. To this end Mr. Nicholls carried out a series of feeding experiments, the results of which are summarized in Table XIX.

TABLE XIX. *Digestion of Living Plankton Organisms by Corals.*

No.	Coral.	Food.	Time fed.	Time for complete digestion.	Remarks.
1	*Favia* (1 polyp)	2 mysids 5 mm. long	9·45 p.m.	$10\frac{3}{4}$ hours	8.30 a.m. digestion completed and remains of mysids embedded in mucus rejected.
2	*Favia* (1 p.)	*Carolinia* 2-3 mm.	9.30 p.m.	Under 13 hours	10.30 a.m. empty shells of the pteropods still enveloped under disc tissue *over* skeleton. Disc contracted away on stimulation exposing skeleton

TABLE XIX.—*Digestion of Living Plankton Organisms by Corals*—continued.

No.	Coral.	Food.	Time fed.	Time for complete digestion.	Remarks.
3	*Favia* (1 p.)	*Cavolinia* 2-3 mm.	9.30 p.m.	Under 13 hours	Ditto.
4	*Favia* (1 p.)	Cumacean 6 mm.	9.30 ,,	,, 13 ,,	10.30 a.m. complete digestion and empty skeleton rejected.
5	*Favia* (1 p.)	Megalopa 3 mm.	10.30 ,,	,, $9\frac{1}{2}$,,	8 a.m. Ditto.
6	*Favia* (30 p.)	Cumaceans 11 mm. 1 to each polyp	11 ,,	,, 10 ,,	9 a.m. empty skeleton lying at bottom of bowl, others in process of removal from surface of colony, a few being rejected from mouths.
7	*Symphyllia* (1 p.)	3 mysids 5 mm.	9.45 ,,	,, $10\frac{3}{4}$,,	8.30 a.m. digestion complete; empty skeletons ejected.
8	*Fungia*	2 mysids 5 mm.	9.45 ,,	,, $10\frac{3}{4}$,,	8.30 a.m. ditto.
9	,,	Mysid 11 mm.	8.55 ,,	,, 12 ,,	8.50 a.m. digestion complete.
10	,,	Mysid 11 mm.	10.10 ,,	,, 9 ,,	7 a.m. ditto.
11	,,	Mysid 10 mm.	8.55 ,,	About 5 ,,	2 a.m. ditto.
12	,,	2 Copepods 3 mm.	8.50 ,,	,, 3-4 ,,	11.45 p.m. digestion practically complete.
13	,,	Crustacea 4 mm.	8.50 ,,	,, 3-4 ,,	11.45 p.m. digestion not quite complete; food reduced to pieces about 0·4 mm. long.
14	,,	Crustacea 9 mm.	8.50 ,,	,, 6 ,,	11.45 p.m. about half digested.
15	,,	Amphipod 4 × 1 mm.	8.50 ,,	,, 4-5 ,,	12.55 a.m. digestion practically complete; cephalo-thorax cleaned out.
16	,,	Mysid 10 × 2	9.0 ,,	Under 8 ,,	5 a.m. digestion probably complete.
17	,,	Mysid 13 × $2\frac{1}{2}$	9.0 ,,	,, 10 ,,	7.20 a.m. digestion already complete. 8.21 a.m. empty skeletons extruded.
18	,,	Mysid 5 × 1	8.50 ,,	,, 12 ,,	12.30 a.m. food on disc; cephalothorax drawn into interior through opening in tissue; abdomen projecting and enveloped with mesenterial filaments. 1.30 a.m. filaments within body of prey. 2.45 a.m. food completely turned round. 3.15 a.m. prey taken in through hole and lying between septa. 4.30 a.m. prey drawn centrally, no longer visible. 8.30 a.m. remains of skeleton found extruded.

The results of these experiments show clearly that the polyps of such typical corals as *Favia*, *Symphyllia* and *Fungia* can digest large plankton organisms with great rapidity,

material caught in the evening being digested and the empty skeletons rejected by the following morning. The process is likely to be quicker under natural conditions, for the experiments necessitated frequent examination, and so exposure to bright light and movement of the digesting corals. The above results establish the fact that the emptiness of the coelenteron so usual in corals during the day is *not* evidence that corals do not feed on zooplankton or that their feeding mechanisms are inefficient.

10. DISCUSSION.

While a full discussion on the nutrition and metabolism of corals must be left to the concluding paper of this series, there are certain matters which may suitably be gone into here. Knowledge on the general process of digestion in corals has been, it is hoped, clarified by the information recorded in this paper. Living animals of suitable size are captured and swallowed with great readiness by corals. Flesh is quickly broken up and dissolved in the coelenteron, the gland-cells in the mesenterial filaments pouring out exclusively a powerful protease whose presence reduces the pH in the coelenteron to about the optimum conditions for the working of the enzyme. Digestion takes place very quickly, and the products of extracellular digestion, consisting probably of small fragments of proteins and of polypeptides, are ingested by the absorptive cells of the mesenterial filament (the locality and manner will be described in the next paper of this series). Within the digestive vacuoles, the process of digestion is carried to completion by proteoclastic enzymes which have their optimum in alkaline media. These enzymes comprise a protease which splits up proteins and polypeptides to amino-acids, and a peptidase which converts only dipeptides into amino-acids. Fat can be digested here and also glycogen, the weak glycogenase having its optimum reaction at about pH 6·5, so that glycogen is presumably digested in the early stages of intracellular digestion when the pH of the vacuoles is about 6·5, final protein digestion being more efficient during the later, alkaline phase. The presence in tissue extracts of an amylase and of a protease having their optima at pH 4·2 and 5·3 respectively is almost certainly due to the very great numbers of zooxanthellae in the tissues. It is significant that in *Physalia*, *Stomolophus*, *Tealia* and *Metridium*, in all of which an amylase has been identified, as noted in the account of literature on digestion in coelenterates, this is in every case accompanied, as is usual in animal tissues, by a maltase. This maltase is absent in *Lobophyllia*. There is also significance in the recent findings of Parker (1928), that glycogen, though *not* starch, will induce a reversal of ciliary current in *Metridium*, in the same manner as meat and various proteins.

Corals, therefore, are as specialized for a carnivorous mode of life in the properties of their digestive enzymes as in the nature of their feeding mechanisms. Protein is digested with great rapidity, fat can be digested slightly, while the only carbohydrate which can be assimilated is that which occurs in animal tissues, namely glycogen. No carbohydrates of vegetable origin can be digested. Since the chief function of carbohydrates (as well as of fats) is to afford energy, it is not difficult to understand why the corals should be among the most highly specialized carnivores in the animal kingdom—for such this study of their digestive enzymes (following that of their feeding mechanisms) proves them to be. The energy requirements of corals are exceptionally low. No energy is required for muscular activity connected with movement ; unlike sedentary lamellibranchs

or brachiopods, there is not even a shell to close or open; there are no complicated circulatory or digestive systems requiring continuous muscular activity for their operation; nor is there any demand for fuel for heat production. Energy is certainly expended during ciliary activity, which is continuous in all corals, but if the theory of Gray (1928) is correct, this does not involve the utilization of carbohydrates, or at any rate does so only in part. Beutler (1929), however, has recently demonstrated the presence of abundant glycogen in the ciliated epithelium of *Actinia equina*. Muscular activity in corals is confined to contracting the polyp, since expansion appears to be the result of the drawing in of water by ciliary activity following muscular relaxation, and to the movements of the tentacles, and in neither case has much opposition to be overcome or much effective work to be done. Further, carbohydrates have not to be stored as in animals, such as Crustacea, which require them for the elaboration of their chitinous exo-skeletons, and though some is doubtless required for the development of the reproductive products, yet reproduction is not the great tax on corals that it is, for example, in lamellibranchs, where great stores of glycogen are accumulated pending the development of the gonads. It is probably not without significance that amylases have been demonstrated in *Physalia* and *Stomolophus*, both free-living, active coelenterates with much greater energy requirements, and in *Tealia* (though not in *Actinia*), where the contractile and feeding movements are greater and more frequent than in corals, and where fixation to the hard substratum requires muscular force.

Protein, on the other hand, is required in great amount for growth, which is rapid in corals, for the replacement of worn tissues and for the formation of the reproductive products, while, after de-aminization (when it may be converted into carbohydrates), it will be available for energy purposes. Carbohydrates have a very minor *rôle* to play in the digestive processes of the Madreporaria.

11. SUMMARY.

1. The coelenteric fluid of *Fungia* has a pH of about 7·8, which drops to about 7·10 after feeding. This is due to the secretion of an extracellular proteolytic enzyme, which breaks down proteins to polypeptides, and has its optimum pH at 7·1.

2. Extracts of the tissues of *Lobophyllia* contain a powerful protease which breaks down proteins and polypeptides to amino-acids and acts best in alkaline media with optimum conditions about pH 9·2, and also a peptidase which acts on dipeptides exclusively.

3. There is a weak intracellular lipase.

4. There is a weak intracellular glycogenase with a pH optimum of 6·5, but no invertase raffinase, maltase, lactase, cellulase or any enzyme acting on glucosides or pentosans.

5. The amylase found in tissue extracts originates in the zooxanthellae. It is absent in extracts of *Dendrophyllia*, a coral without zooxanthellae, but present in the non-digestive, but algal-laden tissues of *Lobophyllia*. It acts on glycogen as well as on starch. Its low pH optimum, at about 4·2, is typical of many plant amylases.

6. The zooxanthellae probably possess a protease with a pH optimum at about 5·3 and a lipase.

7. Corals can digest animal prey, such as planktonic organisms, within the coelenteron in a few hours, the empty skeletons being then ejected. This fact, with their nocturnal feeding habits, accounts for the general absence of food in the coelenteron of corals.

8. The absence of food in the coelenteron is thus not evidence that corals cannot or do not live on animal prey.

9. The Madreporaria are among the most highly specialized carnivores in the animal kingdom, being capable only of digesting animal matter with its constituent proteins, fats and glycogen.

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